Investigating the In Vivo-In Vitro Correlation for the Effect of Kolliphor® EL, ELP and RH40 on the Bioavailability of Fenofibrate in Rats

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ABSTRACT SUMMARY
The effect of three different types of Kolliphor® (Ko); ELP, EL and RH40, on the bioavailability of fenofibrate was tested in vitro and in vivo. The study showed increasing levels of KoELP and KoEL increased the bioavailability of fenofibrate in rats, correlating well with in vitro lipolysis experiments. High levels KoRH40 gave a lower in vivo bioavailability compared to high levels of KoELP and KoEL, most likely due to poor dispersion

INTRODUCTION
Many new drug candidates have poor water solubility, limiting their systemic absorption. To overcome this challenge, many different formulation strategies are used; e.g. the addition of surfactants to increase the dissolution rate of the compound and thereby increase the absorption, or application of lipid based formulation systems containing surfactants.
Kolliphor® ELP (KoELP), Kolliphor® EL (KoEL) and Kolliphor® RH40 (KoRH40) are all nonionic surfactants commonly used in pharmaceutical formulations to improve the bioavailability of poorly water soluble compounds or as emulsifiers in lipid based formulations. KoELP is a purified grade of KoEL, made by reacting castor oil with ethylene oxide in a molar ratio of 1:35, followed by a purification process. KoRH40 is made by reacting 1 mole of hydrogenated castor oil with 40 moles of ethylene oxide.

The purpose of the present study was to examine the in vivo – in vitro correlation for the effect of the commonly used surfactants, KoEL, KoELP and KoRH40, on the bioavailability of poorly water soluble compounds, using fenofibrate as the model compound.

EXPERIMENTAL METHODS

Rat Studies
The animal experiments were approved by the local Animal Ethics Committee. Male Sprague-Dawley rats were divided randomly into 9 groups of 6 animals each. Food was withdrawn 16-20 h prior to the study. The animals were dosed by oral gavage with 2 mg/kg of fenofibrate solubilised with a fixed formulation volume of 10 mL/kg. Blood samples of 0.2 mL were obtained from the tail vein at selected time points after administration. Plasma samples were analysed by reversed phase HPLC using clofibric acid as an internal standard. The animals were allowed access to drinking water 4 h after oral dosing and carrots 10 h after dosing.

Lipolysis Studies
The formulation to digestion media ratio was set to 0.4, to correlate with the ratio between the volume of administrated formulation and the amount of liquid in the rat intestine¹. 10 mL formulation was added into a thermostat-jacketed glass vessel and dispersed for 10 min in 25 mL digestion medium (37 °C). Digestion was initiated by addition of 5 mL pancreatic extract. Sodium hydroxide solution (0.2 M) was automatically added (controlled via the pH-stat controller) to the vessel to maintain constant pH (6.5) during digestion. Samples were taken at 0, 5, 30, 45 and 60 min and immediately treated with lipolysis inhibitor. All samples were centrifuged into two phases; a dispersed aqueous colloidal phase and a precipitated pellet phase. The amount of fenofibrate in both phases was determined by HPLC.

RESULTS AND DISCUSSION

Bioavailability Data
Increasing levels of KoEL and KoELP resulted in increased bioavailability and delayed t\text{max} of fenofibrate (Figure 1). The delay in t\text{max} could be explained in two ways; by a delay in stomach emptying due to intake of higher amounts of digestible material, or by intestinal retention of fenofibrate by entrapment in kolliphor® micelles.
Absorption of fenofibrate from solutions containing KoRH40 showed no difference in t\text{max} with varying surfactant concentration, suggesting KoRH40 is non-digestible, in accordance with previously results². Compared to KoEL and KoELP, KoRH40 led to lower
C_{max} values. For high levels of surfactants (25 %), KoRH40 gave a significantly (p ≤ 0.05) lower bioavailability of fenofibrate.

Figure 1: Average (± SEM) fenofibrin acid plasma concentrations in rats (n = 6) after oral administration of aqueous fenofibrate solutions with varying concentrations of KoELP, KoEL or KoRH40.

**In Vitro Studies**

Lipolysis studies showed that all three types of kolliphor® were susceptible to digestion by enzymes in the pancreatic extract. However, only to a small degree; 25-170 μmol of fatty acids titrated. All three types of kolliphor® was digested to a similar degree in a concentration dependent manner, i.e. more was digested at higher surfactant concentrations.

Figure 2 shows the amount of dose solubilized in the aqueous phase after 60 min of lipolysis. With 2 % KoELP and KoEL up to 57 % of the dose precipitated during the lipolysis experiment. These results correlate well with the *in vivo* data showing increasing fenofibrate bioavailability with increasing kolliphor® concentrations, and suggest that the lower AUC at low surfactant levels is due to drug precipitation in the GI-tract. KoRH40 kept the dose solubilized throughout the lipolysis experiment at all concentration levels, indicating a smaller degree of digestion or higher fenofibrate solubility in the digestion products. As the degree of digestion was found to be comparable across the different types of kolliphor®, the later explanation is more plausible.

Visual inspection throughout the experiment revealed that formulations containing 15-25 % of KoRH40 became poorly dispersed after approximate 40 min of lipolysis. This was not seen for KoEL or KoELP. The lower bioavailability of KoRH40 might therefore be due to poor dispersion *in vivo*.

**CONCLUSION**

Increasing levels of KoELP and KoEL increased the bioavailability of fenofibrate in rats, correlating well with *in vitro* lipolysis experiments.

High levels KoRH40 gave a lower *in vivo* bioavailability compared to high levels of KoELP and KoEL, likely due to poor dispersion.

**REFERENCES**


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